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SOME OBSERVATIONS ON BONE MINERAL IN A CASE OF VITAMIN D RESISTANT RICKETS

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ABSTRACT

Using a new sectioning technique, material from a case of proven vitamin D resistant rickets has been examined, stained and unstained, in the fresh state. Osteoid seams are rapidly and unequivocally evaluated and lined about 90% of all the cortical vascular channels. In addition, a mineralization defect was found surrounding about 25% of the osteocytes present. This is a newly described phenomenon, and it is suggested that it is evidence of a disturbance of osteocyte metabolism.

INTRODUCTION

Cases of vitamin D resistant rickets used to be classified as dwarfism, due to dyschondroplasia, due in turn to an hereditary defect. With the discovery of vitamin D and the recognition of its importance in the formation of the skeleton in postnatal life came the gradual realization that D resistant dwarfs were an unusual form of rickets. Then followed the discovery of incomplete tubular reabsorption of phosphate which in turn led to the discovery that D resistant rickets is a juvenile form of this condition, otherwise known as renal phosphatasia or phosphate diabetes. Subsequent treatment with supplemental phosphate has markedly improved our ability to manage the condition.

The older literature particularly is replete with pathological descriptions of the gross and microscopic anatomical alterations in D resistant rickets. Of late an increasing amount of histochemical information has been added to the established morphology. Little work has been done on the bone mineral in these cases, however, save for microradiography. The reason is two-fold: scarcity of biopsy material and lack of a suitable sectioning technique.

I have developed a sectioning technique which is rapid, economical, simple and allows preparation of perfectly fresh sections of bone without decalcification, embedding, dehydration, work heat or production of significant work trauma. Examination of such material led to the present paper.

Since the features to be described will not be familiar to the average reader, the appearance of normal bone sections such as above will be described first and then the appearance of the sections from the case of D resistant rickets to be described. The reader should realize at the outset that the descriptions will be of features which are due to the presence or absence of mineral in the bone, rather than staining characteristics of the soft tissue, and that the appearance of fresh, undecalcified material is markedly different from that of the routine H&E stain on decalcified and embedded material.

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THE PATIENT

I. K. a 9 year old Caucasian male was first seen at Henry Ford Hospital in March, 1958. Bowing of both lower extremities had first been noted at age 8 months and had been present thereafter, being the reason for his admission to Henry Ford Hospital. The diagnosis of D resistant rickets was made from physical, X-ray and laboratory evidence at the age of 3 years and was subsequently confirmed at different times by a famous eastern and another famous midwestern medical center. He had been on large doses of vitamin D since age 4 years. The patient’s mother and maternal grandfather both have markedly short, bowed lower extremities.

Physical examination revealed marked bowing of femurs, tibias and fibulas. There was minor bowing of the forearms. X-rays revealed widened, irregular epiphyseal lines, irregular thickening of the epiphyseal plates and the aforementioned bowing. Alkaline phosphatase was 16.4 (Bodansky), serum phosphorus 2.0 mg%, serum calcium 9.7 mg%. He had been taking between 50,000 and 100,000 units of vitamin D per day. Tubular phosphate reabsorption was 75% (90% plus is normal).

Osteotomies of both femurs and tibias were done to correct the severe bowing present. The bone removed at this time was reduced to multiple sections by my technique the same day and examined immediately or stained and then examined.

APPEARANCE OF NORMAL BONE

Unstained sections of normal adults from 10-30u thick and mounted either in water or in synthetic resin are quite transparent. With full illumination of the objectives used, there is little detail that can be made out in the bone mineral itself. Partial illumination (due to phase effects) and phase contrast reveal granularity of the various lamellar zones which is due mostly to the mineral present since little granularity remains after decalcification by drawing dilute acid under the cover slip. The osteocytes, canaliculae and Haversian canals are visible due to differences in refractive index between the bone and the mountant. Contrast is much better with water as a mountant than with the standard histological synthetic resins.

If the section be allowed to dry in air or on a warming table, air then fills the spaces of the osteocytes, canaliculae and vascular channels.

An excellent stained preparation can be made by staining the section 48 hours in 1% basic fuchsin, removing the surface stain and mounting. The consistent feature of this stain is that it does not penetrate the walls of the spaces mentioned above unless there is a lack of mineral in the matrix. Most normal adult bone therefore is fuchsin impermeable and the stain will be deposited as a thin layer on the walls of the spaces in the bone. New bone and bone which for one reason or another has lost some of its full complement of mineral will be diffusely permeated by the fuchsin. This interpretation has been proven recently in our laboratories by microradiography done by Mr. J. Parsons.

Where new bone or pathological bone which is fuchsin permeable occurs, there is a continuous spectrum of densities of staining, indicating variations in the amount of fuchsin permeability. However, the decrease from maximum to minimum is not
accompanied by localization of the last remnants around the osteocyte. There is almost no gradient, the stain density decreasing almost uniformly overall.

**D RESISTANT BONE**

Two features of D resistant bone will be described: the osteoid seams and the pericellular defect.

(A) On fresh, water-mounted sections osteoid seams are immediately apparent with the 16 mm. objective as glasslike, hyaline rings lining the walls of vascular spaces. Outboard of the seam where mineralization is in progress and partially completed considerable granularity exists, and the thicker the section the more marked is the contrast between the two. In the present case the seams line about 90% of 348 vascular spaces counted and varied from 10-60μ in thickness. Under polarized light they were distinctly less birefringent than the outlying bone even after decalcification, suggesting that there is a disturbance in collagen formation in the seams. If a section be observed while drying in air, shrinkage of the seam is seen after evaporation of the droplet of water in the Haversian canal. At room temperature the loss of width is 50%; at 40°C on the warming table for 10 minutes it shrinks 75-90% of the original width, retaining its glasslike feature all the time. The mineralized bone outboard of the seam shrinks only 2.2% on cross section, 0.4% on longitudinal with the above treatment.

Sections stained with basic fuchsin as mentioned previously reveal that in every case where an osteoid seam exists the bone immediately outboard of it is fuchsin permeable for a variable distance. The stain density is maximal at the junction of mineralized bone with seam, minimal at the periphery of the osteon. The seam itself takes a homogeneous, dense stain which is not quite impenetrable optically. Destaining 48 hours in tap water differentiates the seam from bone because the former loses much more stain than does the latter.

(B) Unstained, water mounted sections of the biopsy material from the case of D resistant rickets described above reveal a curious dark, granular blur with poorly defined edges surrounding about 25% of 642 osteocytes counted at random. This is a unique phenomenon, not having been observed in the previous 4 years work with sections of this type made from over 100 different patients of all ages, nor in 20 different animals. The granularity under high numerical apertures is seen to be due to multiple fibrillae about 0.2 x 3μ, spiralling in the presumed direction of the collagen bundles in the area.

Allowing the above sections to dry at room temperature under observation reveals that water in the blurred area evaporated from the matrix to be replaced by air. The blurred area (I have coined the term *halo volume* to describe the mineral immediately surrounding osteocytes) is visible with full or partial objective illumination, by phase contrast and on interference microscopy is of less optical density than the surround.

On fuchsin stained material the blur above is stained red. Of incidental interest is the stainability of this halo volume by CoS, Ag, Ag3S, PbS, Alizarin Red S and KMnO4 techniques developed by me.
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DISCUSSION

While it is self-evident that one case does not encompass the total pathology, there can be little doubt that the appearances described are real and probably represent this disease state.

The purpose in describing the osteoid seams was to reveal how easily and unequivocably seams can be detected in fresh, undecalcified, undehydrated material. Since it takes about 3 minutes to make and mount such a section, the method offers considerable promise in clinical biopsy situations where an osteomalacic process is suspected. It is well known that the demonstrability of osteoid seams by the standard H & E decal techniques is uncertain. Minor variations in the total technique may alter the difference in staining sought for as a means of identification. There is on the other hand no uncertainty in the fresh, undecalcified material.

Numerous counts of seams in material from patients of all ages without an osteomalacic process reveal that in the healthy adult the incidence of seams is about 3 per 1000 osteons. In children the count ranges considerably higher, up to 100 per 1000. Accordingly, the 900 per 1000 present in I. K. is markedly elevated.

A final note of interest is the poor birefringence of the osteoid seams in their wet state. This is characteristic of seams in general, not just in the present case, and suggests very strongly that the seam is not normal matrix but is lacking in total collagen content or in the organization characteristic of collagen in normal bone matrix. This in turn suggests that the seam is the result of a metabolic disturbance in the cells forming it rather than the result (only) of mineral deficit.

The fuzzy blur around 25% of the osteocytes in the present case of D resistant rickets is intriguing. The microscopic observations leave no room for doubt about the reason: there is incomplete mineralization in the affected volume of bone. Since standard H & E decal sections made of the same case reveal some excessive basophilia but not lack of matrix in this volume, a quantitative lack of matrix is unlikely. A qualitative change is probable, however.

It is widely assumed that the mineralization defect in rickets is due solely to lack of mineral elements in the blood and that there is no primary metabolic disturbance in the osteoblasts or osteocytes. The pathological halo volumes present here can however be interpreted in the opposite manner. It is possible that the enzyme defect that makes the renal tubular cell unable to remove phosphate from the glomerular filtrate is more wide-spread and affects other cells including the osteocyte. It is certain that if a mineral deficiency alone were the cause of the mineralization defect in rickets the parts of the bone farthest from the cells, not closest to them, should be most affected, since passage of mineral from the canaliculae, through the cell and into matrix indicates that of necessity the concentration gradient must be positive, that is, maximal at the cell, minimal at a distance from it.

The mineralization gradient in fact is negative. This could be interpreted to mean that some metabolic product of the osteocyte in D resistant rickets prevents mineralization from occurring close to the cell where its concentration is maximal.
In other words, I suggest that there is a primary, cellular metabolic defect in this disease which involves the osteocyte. This defect may involve phosphate metabolism also but not necessarily so.

**BIBLIOGRAPHY**

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Figure 1

Cross section ulna undecalcified, 20 yr. male, 700X, N. A. -0.95 Basic Fuchsin. Three osteocytes and parts of some of their canaliculae are in focus. Stain does not penetrate bone walls of physiologic spaces. The canaliculae are so small (0.4u diam.) that they are defined with difficulty.
Cross section humerus 20 yr. male. 1000 x, N.A. 1.32-undecalcified. HgS stain. Osteocyte lacuna and parts of canaliculae close to it are stained by virtue of HgS precipitated in bony wall of the spaces. Penetration of the wall about 1.0 u. CoS, Ag, Ag₃S, PbS, KmnO₄ and Alizarin Red S produce similar stains.
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Figure 3

Cross section tibia. I.K. (subject of text). 300 X, bright field, N.A. -0.65, 50% illuminated. Unstained, water mount. The osteoid seam is the hyaline ring surrounded by granular, partly mineralized bone. Section 30μ thick. Contents vascular canal avulsed by section preparation.
Cross section tibia, I.K. unstained, undecalcified, 100X, bright field N.A. -0.65 reduced 50%. The fuzzy blur referred to in text can be seen affecting 5 osteocytes to a marked degree, 6 to a lesser degree. Normally mineralized bone never shows this phenomenon.
Figure 4 B

Longitudinal section opposite tibia same optical factors. Section dried at room temperature. The granularity in the blurred zones surrounding many of the osteocytes is more apparent due to accentuation of differences in refractive index by using air as a mountant.
Figure 5

Cross section, I.K. undecalcified, 1000 X, N.A. 1.32, full illum, stained with CoS. Compare with Figure 2. The cobalt has diffused widely beyond the confines of the two osteocyte lacunae in the centers which are blacked out. This is a pathological “halo volume”.

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